Comparative analysis of plant-based high-protein ingredients and their impact on quality of high-protein bread

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Abbreviations: HPI, high-protein ingredient; TDF, total dietary fibre; %DM, percentage based on dry matter; FWA, Farinograph water absorption; TM, Torque maximum; PMT, peak maximum time; PV, peak viscosity; FV, final viscosity; DF, damping factor; SV, specific volume; CA, correlation analysis; cc, correlation coefficient; PCA, principal component analysis; proteinE, percentage of calories provided by protein

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Abstract

The orientation of consumers and industry towards plant-based foods on one hand and high-protein products on the other is persistently increasing. Bread, as a staple food, is a promising matrix for the incorporation of plant-based high-protein ingredients to combine both trends. This study aims to provide a better understanding of techno-functional changes and impacts of plant-proteins during bread production, which could advance the development of high-quality products with high levels of plant-protein. A selection of high-protein ingredients from wheat, maize, potato, carob, pea, lupin and faba bean were subjected to compositional analysis and applied in wheat bread formulations, replacing 15 % of wheat flour. Their impact on dough properties (gluten-aggregation, pasting behaviour, rheology) as well as bread quality (volume, crumb structure, crumb hardness) was analysed. The high-protein ingredients were found to affect gluten-aggregation, pasting and bread characteristics. Results indicated a weakened gluten-network in doughs containing potato and pea protein. Also pasting behaviour was mostly affected by the potato protein suggesting a heat induced improvement of its baking performance. Good bread quality, represented by high specific volumes and low crumb hardness, was observed for gluten, zein and carob. Breads with pea, lupin and faba bean showed only slightly inferior quality characteristics.

1. Introduction

Plant proteins are a growing market in the food sector and represent an opportunity to meet nutritional needs of the growing world population, while at the same time realising the transition to a more sustainable food production. Thus, the demand from consumers, industry and governmental institutions for high-quality food with high contents of plant-protein is substantial. Bread plays an important role in the human diet and relatively large amounts are consumed worldwide (Henchion et al., 2017). It is therefore a promising matrix for the incorporation of plant-protein ingredients in order to supply a broad range of customers with plant-based high-protein food. Definitions and requirements for the attribute 'high-protein' can be found in many regulations all over the world and they vary greatly. According to regulation (EC) No 1924/2006, a product qualifies for the claim 'high in protein' if 20 % of its calories are provided by proteins. In order to reach this protein content while maintaining adequate product quality, plant-based ingredients with high protein contents can be utilised. Protein ingredients from plant sources are usually classified by their protein content: as flours (protein $<$ 65 %DM), concentrates (protein $>$ 65 %DM) and isolates (protein $>$ 90 %DM) (Boye et al., 2010). However, the terminology is sometimes misleading since especially isolated legume protein products often do not reach a protein level of 90 %DM but are still considered isolates regarding their production procedure (Arntfield and Maskus, 2011). Hence, this study will focus on the generalised term high protein ingredients (HPIs). The partial substitution of wheat flour for bread production by HPIs from numerous sources has been previously investigated, including dairy proteins (Kenny et al., 2000); cereals

(Bugusu et al., 2002), pseudo-cereals (Sanz-Penella et al., 2013) and legumes (Villarino et al., 2015; Turfani et al., 2017; Marchais et al., 2011). However, these studies are usually focused on one type of plant-protein. This study aims for a comparison of HPIs from different plant sources and investigates their impact on wheat bread formulations and their suitability to produce high-protein quality breads. The selected HPIs are the cereal proteins gluten (wheat) and zein (maize), potato protein, and proteins from the following legumes: carob (*Ceratonia siliqua*), pea (*Pisum sativum*), lupin (*Lupinus angustifolius*) and faba bean (*Vicia faba*). While some of these ingredients are commercially available and well characterised, others are produced from emerging protein sources and their properties and potential for bakery applications need to be explored. Wheat-gluten can be described as a group of proteins, which are mainly responsible for the structure-forming ability of wheat flour during dough production and baking. Gluten has unique viscoelastic properties, protein-protein interactions, water holding capacity and thermosetting characteristics (Day, 2011). It is widely used as bread improver in the baking industry. Zein is the prolamine fraction originating from maize-proteins. Amongst other cereal-prolamines, it exhibits the highest content of hydrophobic amino acids and thus a very high protein hydrophobicity and low water solubility (Belitz et al., 1986). Potato proteins can be obtained from the side-product potato juice from potato starch production and therefore represent an economically promising ingredient for plant-based high-protein foods. Previous studies report high water solubility and indicate outstanding techno-functional properties including heat induced gelling (Alting et al., 2011). Legumes are generally accepted to be beneficial with regard to environmental aspects, due to their ability to fix nitrogen, and from a nutritional perspective (Henchion et al., 2017). While cereal proteins exhibit high contents of sulphur-containing amino acids and are low in other essential amino acids (e.g., lysine), legumes represent the opposite and are therefore favourable for incorporation in cereal products to balance the amino acid profile and increase protein quality (Henchion et al., 2017). Important protein ingredients from legumes are protein-rich flours (obtained by dry-processing) and isolates (produced by wet-processing). Dry-processed (i.e., air-classified) products exhibit native protein characteristics and lower protein contents, whereas protein isolates have a higher protein-purity and altered properties conditioned by the isolation procedure (Schutyser et al., 2015). Furthermore, isolates mainly consist of the protein fractions albumins and globulins. An exceptional legume protein is carob germ protein, which contains a high glutelin-fraction (about 68 %) called caroubin. This protein has been reported to have gluten-like properties (Smith et al., 2010; Feillet and Roulland, 1998). The objective of this study is to characterise protein-rich fractions of a wide range of plant sources and to evaluate their impact on dough and bread quality characteristics.

2. Experimental

2.1. Material

Five commercially available high-protein ingredients (HPIs) were used in this study. Potato protein isolate (Patissionate 306 P) was obtained from Avebe, the Netherlands; pea protein isolate (NUTRALYS PEA BF) from Roquette, France; carob germ flour (GRINDSTED VEG PRO S1) from

Danisco, UK; vital gluten (NUTRALYS W) from Roquette, France; and corn protein (Zein) from Flo Chemical Corporation, Massachusetts, US. Additionally, two HPIs: blue lupin protein isolate and faba bean flour (fine fraction, protein-rich) were experimentally produced and provided by Fraunhofer Institute IVV, Freising, Germany. Wheat flour was supplied by Whitworth Bros Ltd, UK; dry yeast (4 % moisture, 50 % protein, 5 % fat, 40 % carbohydrates) by Puratos, Belgium; salt by Glacia British Salt Ltd, UK; and vegetable oil by Musgrave, Ireland. Chemicals were purchased from Sigma-Aldrich (Missouri, USA) unless stated otherwise.

2.2. Compositional analysis

HPIs and wheat flour where subjected to compositional analysis including determination of protein (nitrogen-to-protein conversion factor 6.25, based on MEBAK 1.5.2.1), dry matter/moisture (air-oven method at 130 °C until constant mass reached), ash (incineration in muffle furnace at 550 °C for 5 h, charred prior to muffling using open flame, based on AOAC 923.03) and fat (Soxhlet method using SoxCap and Soxtec (Foss UK Ltd, UK)), digestion with 4 M HCl prior to extraction, based on AACC 30-25.01). The analysis of total dietary fibre (TDF) was performed by Concept Life Science Ltd in accordance with AOAC 991.43. The carbohydrate value was obtained by subtraction (i.e., 100%−[protein%+moisture%+ ash%+fat%+TDF%]). Total starch content was analysed using the enzyme kit K-TSTA supplied by Megazyme, Ireland. All values, except the moisture content, are expressed as percentage based on dry matter (%DM).

2.3. Empirical dough analysis

Properties of wheat flour and mixtures of wheat flour and HPIs (HPI/flour blends) in a ratio of 85 % to 15 %, respectively, were analysed. Moisture contents of blends were calculated considering the determined moisture contents of wheat flour and HPIs according to their ratios.

2.3.1. Farinograph

Water absorption (FWA) was determined by Farinograph (Brabender GmbH and Co KG, Duisburg, Germany) water absorption (FWA) was determined following AACC 54-21.02. Titration trials were performed adjusting doughs from wheat flour and HPI/flour blends to a consistency of (500 ± 20) Farinograph units (FU).

2.3.2. GlutoPeak test

Gluten-aggregation properties of wheat flour and the HPI/flour blends were investigated using the GlutoPeak (Brabender GmbH and Co KG, Duisburg, Germany). This device applies high shear to a flour/water slurry. 9 g flour (based on 14 % moisture; adjustments according to AACC 82-23.01) were added to 9 g of deionised water (36 °C) weighed into the sample cup (adjusted to reach a slurry weight of 18 g). The measurement was started immediately after a brief premixing step. The paddle speed was set to 2750 rpm and water circulating through the jacketed sample cup maintained the sample temperature at 36 °C. The torque reading was recorded over time. The maximum test time was set to 10 min and measurements were stopped approx. 30 to 50 s after detection of the major peak. The curve was evaluated by the software provided with the instrument regarding Torque Maximum

(TM, expressed in Brabender units BU) and Peak Maximum Time (PMT, expressed in s).

2.3.3. Rapid visco analysis

The pasting behaviour was examined using Rapid Visco Analysis (RVA Super 3, Newport Scientific, Warriewood, Australia) following AACC 76-21.02. A heating profile was applied: equilibration at 50 °C for 1 min, heating to 95 °C at 0.2 °C/s, holding at 95 °C for 162 s, cooling to 50 °C at 0.2 °C/s, maintaining at 50 °C for 120 s. The pasting variables peak viscosity (PV), setback and final viscosity (FV) were determined from the viscogram using manufacturer-supplied software.

2.4. Recipe adaptation and bread production

Bread samples were prepared according to the formulation in Table 1. For incorporation of HPIs, 15 % of wheat flour were replaced. Water levels where applied as determined by Farinograph trials - FWAs (Table 3). Baking properties of the HPIs were evaluated by preparation and examination of micro-scale bread loafs (based on 65 g of dough). The straight dough method was applied. After activating the yeast by dissolving in 30 °C tap water for 10 min, the yeast suspension was added to the remaining, previously weighed ingredients. A total dough amount of 630 g was prepared by mixing with a Kenwood Chef (Kenwood Manufacturing Co. Ltd., UK) kitchen machine at speed 1 and 2 for 1 and 7 min, respectively. After dividing the dough into nine pieces of 65 g ± 1 g, the pieces where moulded (very sticky doughs were shaped with a dough scraper), put into baking tins and proofed for 75 min at 85 % humidity and 30 °C (KOMA BV Sunriser, Reormond, the Netherlands).

Baking was performed in a deck oven (MIWE Condo, Arnstein, Germany) at 210 °C top and bottom temperature for 14 min with steaming the baking chamber prior to loading with 700 ml and open draft throughout the whole baking process. Breads where removed from tins and left on a grid at ambient temperature for 1 h to cool down. The presented values represent the mean of three independently performed baking trials.

2.5. Dough rheology

Dough was produced according to the procedure reported in section 2.4, but yeast was omitted. Dough was allowed to rest for about 10 min before measurements. Viscoelastic properties of doughs were examined by using a stress/strain-controlled rotational rheometer (MCR 301 Anton Paar, GmbH, Germany) equipped with a PP50 parallel-plate measuring system (serrated surface to avoid slippage). The lower plate was set to 30 °C. After positioning 10 g of dough between the plates, the upper plate was lowered to 1.025 mm, the sample trimmed and a layer of mineral oil applied to prevent desiccation. The final gap was set to 1 mm prior to starting the test. The rheological variables storage modulus (G'), loss modulus (G'') and damping factor ($DF = tan\delta = G''/G'$), describing viscoelastic dough properties, were obtained from frequency sweeps (ω = 100 - 0.1 Hz; data obtained at angular frequency of 2.54 Hz for control and 2.58 Hz for HPI doughs, respectively) A constant strain of 0.01 % (linear viscoelastic range previously determined by amplitude sweeps) was applied.

2.6. Bread characteristics

Specific volume (SV) was measured with a Volscan Profiler (Stable Micro Systems, Surrey, UK). To analyse crumb structure and hardness, three slices (25 mm) were cut out of the middle of each of 3 loaves. A C-Cell Imaging System (Calibre Control International Ltd, UK) was used to capture images of the slices and to determine the variables: number of cells and area of cells. Crumb hardness was analysed with a TA-XT2i Texture Analyser (Stable Micro Systems, Surrey, UK) equipped with a 25 kg load cell. A 20 mm cylindrical probe was used to compress the centre of the slice to 40 % of its height as part of a texture profile analysis (TPA): test speed 5 mm/s, post-test speed 10 mm/s, trigger force 0.05 N, waiting time between compressions 5 s. Lightness of crust (L^*) was measured by a Colorimeter CR-400 (Konica Minolta, Japan) using the CIE $L^*a^*b^*$ colour space.

2.7. Statistical analysis

All measurements were performed in triplicate. Data analysis was carried out using R (version 3.5.1). One-way ANOVA with post-hoc pairwise Tukey test was used to show significant differences ($p < 0.05$). A correlation analysis (CA, significance level $p < 0.05$) determining correlation coefficients (cc) as well as a principal component analysis (PCA) was conducted using the main technological properties of doughs and breads to evaluate and visualise correlations and identify groups among control and HPI formulations.

3. Results

3.1. Composition

The protein content determined for wheat flour is 14.09 %DM (Table 2). High-protein ingredients show values at least 3.5 times higher. The lowest levels were obtained for carob (55.04 %DM) and faba bean (61.25 %DM). Pea and gluten contain 80.19 %DM and 83.11 %DM protein, respectively, representing the medium-protein-level HPIs. Zein, potato and lupin show protein values over 90 %DM ranging from 91.79 %DM for zein to 94.51 %DM for lupin. In spite of different protein levels, the authors decided for a constant replacement of 15% wheat flour in order to reach a comparable dilution of wheat gluten and starch. The determined fat content of gluten, potato and carob was <1 %DM. Wheat flour (1.97 %DM), zein (2.66 %DM), lupin (2.94 %DM), faba bean (3.81 %DM) and pea (6.45 %DM) are slightly higher in fat. Potato, gluten and zein show low ash contents between 0.05 %DM (potato) and 1.16 %DM (zein), similar to wheat flour with 1.09 %DM. In contrast to this, ash levels of faba bean, lupin, pea and carob range from 5.43 %DM (faba bean) up to 7.04 %DM (carob). Total dietary fibre accounts for 2.88 %DM in zein, 2.30 %DM in wheat flour and less in the other HPIs. Carob however contains 17.67 %DM of fibre. A total starch content of 72.38 %DM was found for wheat flour. HPIs contain less than 0.2 %DM of starch; except gluten and faba bean, which show starch values of 4.95 %DM and 7.77 %DM, respectively. In order to interpret the protein levels of control and HPI formulations, percentages of calories provided by protein (proteinE) were calculated. This was achieved by taking into consideration the compositional data of all ingredients (Table 2) as well as the ingredient ratios in the formulations (Table 1). Protein accounts only for 14.48 % of calories in the control formulation. All HPIs formulations show values higher than 20 % ranging from 21.23 % (carob) to 26.64 % (lupin).

3.2. Empirical dough analysis

3.2.1. Farinograph

In wheat dough production, the amount of water added and the resulting dough consistency are important parameters to allow sufficient distribution and hydration of dough materials and gluten-network development. The obtained FWAs (Table 3) show significant differences and indicate different water absorptions of HPIs and different abilities to compete for water with wheat flour. For wheat flour a FWA of 63.0 % was determined. Except for zein (60.8 %) and faba bean (62.2 %), all HPI/flour blends absorb significantly more water than wheat flour. A moderate increase was observed for potato (65.2 %) and lupin (66.2 %). Carob, gluten and pea caused the highest FWAs accounting for 69.8 %, 70.2 % and 71.7 %, respectively.

3.2.2. GlutoPeak test

During GlutoPeak measurements, a high speed rotating element subjects the sample slurry to an intense mechanical action. This allows gluten-aggregation and network-formation. The result is an increase in recorded torque, followed by a decline due to a destruction of the network by further mixing. Considering the small sample sizes and short measuring times, the GlutoPeak test is ideal to provide valuable information about rheological properties of HPI/flour blends. Measurements for the gluten formulation could not be performed with the reported method due to exceeded maximum torque of the device. Figure 1B schematically shows a typical GlutoPeak curve of wheat flour for comparison and explanation of variables. The torque curve observed for wheat flour (Figure 1A) slightly rises and reaches the equilibrium plateau in an initial phase. The following strong increase in torque reaches the TM of 74 BU at a PMT of 53 s. The replacement of 15 % of wheat flour by HPIs leads to aggregation profiles entirely different to the typical curve shape. Significantly different PMTs and TMs were obtained. Pea shows an immediate increase in torque within the first few seconds of the test and is lacking the equilibrium plateau. The TM of 83 BU is reached after 16 s in form of a sharp peak. After the maximum, a small decrease of torque was observed. In contrast to this, zein exhibits a curve with a small equilibrium plateau at the start and two broad peaks. A TM of 48 BU was detected after 105 s. The overall curve shape of carob with two broad peaks and a slightly pronounced equilibrium plateau is similar to zein. However, the curve obtained for carob appears much more condensed, TM is higher (58 BU) and PMT lower (41 s). The aggregation profiles of lupin and faba bean both exhibit a slightly pronounced equilibrium plateau and a sharp peak, followed by a slight decrease in torque and a small shoulder. The TM determined for lupin is with 59 BU higher than for faba bean (51 BU). Peak maximum times accounting for 34 s (lupin) and 33 s (faba bean) and are not significantly different from each other. The GlutoPeak curve of potato is lacking the equilibrium plateau. Torque increases immediately and reaches the maximum of 54 BU after 17 s which is pronounced as a steady state rather than a sharp peak. It is followed by a very strong decrease in torque.

3.2.3. Rapid visco analyis

Rapid visco analysis of wheat flour and HPI/flour blends was performed to investigate the impact of HPIs on pasting properties and their behaviour during heating. Significant differences were observed amongst the HPI/flour blends and compared to wheat flour (Table 3). Except for potato, all HPIs lead to a decrease in PV compared to wheat flour with 2390 cP. The lowest PVs from 1415 cP (zein) to 1540 cP (lupin) were obtained for zein, lupin, pea and carob. Slightly higher PVs (but smaller than wheat flour) were measured for gluten (1683 cP) and faba bean (1852 cP). Only the HPI/flour blend containing potato showed no significant difference in PV compared to wheat flour. Setbacks of all HPI/flour blends are lower than for wheat flour (1282 cP). However, potato (1058 cP), faba bean (1158 cP) and lupin (1161 cP) show setbacks in the same range as wheat flour. The other HPIs exhibit much smaller values between 680 cP (pea) and 877 cP (zein). Determined FVs range from 1589 cP (pea) up to 2544 cP (wheat flour). Final viscosities of all HPI/flour blends were lower than for wheat flour (2544 cP). While potato has a PV similar to wheat flour, it shows a significantly lower FV.

3.3. Dough rheology

Control and HPI doughs (full recipe, yeast omitted) were subjected to oscillatory tests in order to obtain information about their viscoelastic properties. A DF of 0.401 (Table 3) was obtained for the control. All doughs containing legume HPIs have a significantly lower DF. The decline is more pronounced for pea (0.325), carob (0.327) and faba bean (0.329) than for lupin (0.349). The lower DFs represent a more elastic behaviour of these doughs compared to the control. The DFs of zein (0.387) and gluten (0.385) are not significantly different from the control. The highest DF was observed for potato, representing the sample with the most viscous behaviour.

3.4. Quality characteristics of breads

A visual evaluation of the breads (Figure 2) reveals striking differences in colour, size and crumb structure. This observation was confirmed by determination of bread quality characteristics. The SV of the control bread is 2.55 ml/g (Table 3). The gluten bread reaches a SV of 3.81 ml/g, which is higher than the SV of all other breads. Breads from lupin (1.98 ml/g) and pea (2.00 ml/g) are smaller than the control; breads containing faba bean (2.26 ml/g), zein (2.63 ml/g), carob (2.73 ml/g) and potato (2.81 ml/g) are not significantly different from the control. However, significant differences were observed within the latter group and between this group and the smaller breads with lupin and pea. The results for crumb structure variables show few significant differences. The number of cells ranges from 1543 (faba bean) to 1902 (gluten). The cell areas lie between 44.5 % (pea) and 49.0 % (gluten). Potato, faba bean and gluten exhibit larger cells than the pea bread. With a hardness of 4.12 N, the gluten bread is softer than the control (11.81 N). In contrast to this, potato (19.02 N) and lupin (20.11 N) lead to a harder crumb. Carob (7.84 N), zein (15.10 N), faba bean (20.11 N) and pea (16.68 N) breads are not significantly different in hardness compared to the control. The darkest crust was observed for faba bean (57.07) and the lightest for zein (72.80). Crusts of the control (71.84) and the potato (70.58) bread exhibit a lightness similar to the zein bread. Carob, lupin, gluten and pea breads represent moderate L* values ranging from 62.33 (carob) to 66.83 (pea).

3.5. Principal component analysis

A PCA was performed based on selected dough and bread characteristics. The first Dimension mainly represents hardness, cell area, number of cells and SV, whereas Dimension 2 models a measure of DF, PV and FV. Hierarchical classification was used to divide the formulations into 5 groups. Cluster A in the upper half of the diagram represents the control and potato formulation and is characterised by high PV and FV. Clusters B and C in the bottom left comprise formulations containing legumes which exhibit low DFs, SVs and higher hardness. Zein and carob formulations represent cluster D with low PV and FV but medium SV and hardness. The gluten formulation forms cluster E and is clearly separated from the other groups by its very high SV and area of cells as well as low hardness.

4. Discussion

The objective of this study is to evaluate and compare the performance of different HPIs in wheat bread formulations and their potential for the production of high-protein breads. The proteinE was calculated based on compositional results and exceeds 20 % for all HPI formulations in this study (Table 2). Compositional changes occurring during proofing and baking (i.e., starch degradation, sugar consumption by yeast, denaturation and degradation of proteins (Rosell, 2011)) will slightly change the proteinE of breads compared to formulations. Nevertheless, the values suggest a great potential for these formulations to produce high-protein breads in accordance with regulatory requirements in Europe. Next to legal compliance, an understanding of the effects leading to different qualities of high-protein breads is important for further product prototyping. The proteins present in the HPIs are likely to affect the characteristics of the formulations depending on their structural and functional properties and by potential interactions with wheat flour components (protein impact). Additionally, the replacement of 15 % of wheat flour leads to a dilution of the technologically most important flour components gluten and starch (dilution effect); and the presence of minor components in the HPIs can also influence dough and baking characteristics.

4.1. Water absorption

One major impact of the HPIs on the formulations is a change in their water absorption to reach a certain consistency. In spite of the dilution effect on gluten and starch, an overall trend towards increased FWAs was observed because HPIs mainly contain proteins, which absorb water themselves. The partial substitution of wheat flour by legume protein isolates or flours, namely carob, pea and lupin, has been reported to increase FWAs (Turfani et al., 2017; Marchais et al., 2011; López, 2014). An increase of protein content in wheat flour by 1 % is expected to cause an average FWA increase of 1 % (Sluimer, 2005). This is in accordance with the FWA increase of 7 % for the gluten/flour blend, whose protein content is 9.6 % higher than for the wheat flour. However, a general correlation between total protein contents of HPI/flour blends and FWAs could not be observed (cc: 0.19). In contrast to the general trend, zein caused a FWA decrease. This can be explained by its high insolubility combined with a very high number of hydrophobic amino acids and overall protein hydrophobicity (Belitz et al., 1986) leading to a low water absorption. No significant change in FWA was observed for faba bean. This difference, especially compared to the other legume HPIs, might be due to its relatively low protein content and its production procedure. Air-classification of legumes, compared to wet protein isolation procedures, usually produces ingredients with higher solubility (Schutyser et al., 2015), which could also lead to lower water absorption.

4.2. Impact of HPIs on gluten-aggregation

While GlutoPeak measurements have been utilised to investigate wheat flour quality and differences in wheat protein properties, they, to the best of our knowledge, have not been used to determine aggregation behaviour of wheat flour blends containing non-wheat HPIs. High-quality wheat flours with higher protein contents (i.e., higher gluten contents) usually exhibit earlier and higher peaks than weak flours in GlutoPeak curves due to a stronger gluten-network (Amoriello et al., 2016). Based on this, measurements with HPI/flour blends are expected to show lower and later peaks compared to the wheat flour (dilution effect). Even though the results in this study do show an overall tendency towards lower TMs, the majority of the peaks exhibit earlier instead of later PMTs (except zein). This suggests that there are other factors affecting the gluten-aggregation in HPI/flour blends. According to Bouachra et al. (2017), who reported an impact of lactic acid on GlutoPeak curves, the presence of (positive) charges in the sample influences gluten-aggregation. An increased number of charges leads to stronger intramolecular repulsive forces which promotes faster unfolding of gluten molecules and therefore accelerates gluten-aggregation. At the same time, the increased presence of charges in the sample causes a more rapid breakdown of gluten because of higher intermolecular repulsive forces and the hindrance of the formation of new bonds. The applied HPIs differ in their solubility and content of charged amino acids, which are both factors to increase the number of available charges in the sample slurry. Legumes and potato exhibit high contents of charged amino acids; additionally, potato proteins are highly water soluble (Boye et al., 2010; Arntfield and Maskus, 2011; van Gelder and Vonk, 1980; Alting et al., 2011). Zein on the other hand, contains only few charged amino acids and has a very high overall hydrophobicity (Belitz et al., 1986). In accordance with the present results, the content of charged amino acids in combination with protein solubility seems to be the major influence of HPIs on PMTs. A flour with a fast build-up of gluten-network, sharp gluten-peak and rapid breakdown is considered weak, whereas a longer PMT seems to allow for a fully developed and more stable gluten-network (Goldstein et al., 2010). This implies a relatively weak gluten-network with pea and potato; medium strength with faba bean, lupin and carob; and high strength with zein. The TM appears to be mainly related to the water absorption of HPIs. A good correlation between FWA and TM was found (cc: 0.86) when comparing HPI/flour blends (wheat flour exempt because of different gluten content). Furthermore, interactions between non-wheat proteins and gluten can occur and affect gluten-aggregation. Curves of all HPI/flour blends (except potato) exhibit a more or less pronounced second peak. This could be caused by interactions between gluten and HPI proteins subsequent to gluten-aggregation and during breakdown, delaying a rapid torque decrease. In general, synergistic interactions between different food proteins forming a stronger coaggregated network have been previously reported (Lin et al., 2017). Bugusu et al. (2002) showed interactions between zein proteins and gluten in wheat dough systems using confocal microscopy after fluorescence labelling of the proteins. Feillet and Roulland (1998) described caroubin, the main protein fraction in carob, as a gluten-like protein with similar rheological properties and similar protein interactions. Since zein and carob are the HPIs which lead to the most pronounced second peak in the GlutoPeak curve, a synergistic network-formation of these proteins with gluten can be hypothesised.

4.3. Impact of heat treatment on HPI/flour blends

The behaviour of a dough and its constituents during and after heat treatment is of major importance with regard to the baking process. Rapid visco analysis provides information on heat induced changes in viscosity of flours suspended in excess water. In wheat flour samples, it is mainly a tool to investigate starch gelatinisation properties. In HPI/flour blends, the starch content is substantially reduced (dilution effect). Less available starch in the samples leads to lower viscosities. This is in accordance with the generally lower PVs observed for HPI/flour blends compared to wheat flour. Only for gluten and faba bean, which contain considerable amounts of starch (4.95 % and 7.77 %, respectively), the decrease in PV is less pronounced. A positive correlation between the calculated sample starch content and PV was detected (cc: 0.950; potato exempt). Also López (2014) reported a decreased PV in RVA when partially replacing wheat flour by plant-protein isolates. Marco and Rosell (2008) observed the same trend in rice-flour systems. The results of the present study suggest a unique pasting behaviour of potato protein in mixture with wheat flour. In spite of a much lower starch content, a PV as high as for wheat flour and a relatively low Setback and FV were measured. Marco and Rosell (2008) proposed an impact of heat induced gelation of non-wheat proteins on PV and setback of rice flour/plant-protein isolate mixtures. Although literature on gelling behaviour of potato proteins is scarce, some of the protein fractions (e.g., patatin) have been reported to form strong gels with properties similar to ovalbumin and beta-lactoglobulin (Alting et al., 2011).

4.4. Viscoelastic dough properties as affected by HPIs

The DFs reveal a clear grouping of the samples by botanical source of the proteins. All legume proteins lead to lower DF, doughs with cereal HPIs exhibit DFs similar to the control, and the potato protein increases dough DF representing a larger viscous proportion than the control. However, small-deformation properties, as measured by oscillatory tests, do not necessarily represent large-deformation properties and performance in baking (Sliwinski et al., 2004). The fact that the potato dough shows the highest DF can be related to its rapid gluten-breakdown observed in GlutoPeak measurements. The constant mixing time applied for all recipes might have exceeded the mixing tolerance of gluten in the potato formulation causing a lack of elasticity and a higher viscous proportion.

4.5. Quality characteristics of high-protein breads

Gluten is known to improve SV and used as an additional ingredient in wheat bread recipes (Arendt and Zannini, 2013). The replacement of 15% of wheat flour by gluten leads to breads expectedly high in quality characterised by the highest SV and the lowest hardness in comparison to all other formulations. The legume breads from pea, lupin and faba bean show tendencies towards lower SV and higher hardness compared to the control. The partial replacement of wheat flour by pea protein isolate (Marchais et al., 2011) and lupin flour (Villarino et al., 2015) has been previously reported to decrease SVs and increase crumb hardness. Specific volume and crumb hardness of faba bean were not significantly different from the control, which suggests an adequate quality. Carob and zein breads show better overall quality characteristics than the control. This is consistent with the findings from Turfani et al. (2017) who reported higher bread volumes with composite wheat/carob flour; Bugusu et al. (2002) observed the same trend for breads from composite wheat/zein flours. Interactions and potentially synergistic network-formation have been suggested by GlutoPeak results for carob and zein. Due to the rapid gluten-breakdown observed in the GlutoPeak test and the high viscous proportion of the dough (high DF), potato was expected to show poor bread quality. However, a high SV but also a high hardness were measured. As suggested by RVA, the potato protein is substantially affected by heat treatment. This seems to have a positive impact on expansion properties of the dough during baking. The high hardness might be caused by a competition of gelling protein and gelatinising starch for water during heating. Thus, less starch would be gelatinised and a higher initial hardness of the product would be observed. A hydration depletion of starch has been previously reported to increase crumb hardness by Martínez et al. (2018). Crust browning in breads is the result of caramelisation of sugars and the Maillard reaction. Components involved in those reactions are carbohydrates, proteins and water (Purlis, 2010). No correlation between L^* and protein content of the formulations was detected (cc: 0.04). This might be related to the fact that the HPIs contain varying amounts of carbohydrates and lysine, which is an important source of primary amines for Maillard reaction in proteins (Purlis, 2010) and therefore substantially supports browning.

4.6. Effects of minor components

The values for carbohydrate contents of HPIs in this study were obtained by calculation. Considering the determined contents of total starch and preliminary results for Fructose, Glucose, Maltose and Glucose (data not shown), the values suggest considerable amounts of non-starch carbohydrates and fermentable sugars present in some of the HPIs; faba bean, carob, gluten and pea in particular. Additional to its effect on crust browning, this can have an impact on yeast activity and SV of the breads. Carob leads to breads with high quality characterised by a high SV and low crumb hardness. Next to its protein properties, also its high fibre content can be partly responsible for this result. Carob seeds are the raw material for the production of locust bean gum. This hydrocolloid has been reported to improve baking properties and loaf volume (Azizi and Rao, 2004). Even though germ and endosperm, which is the source of locust bean gum, are separated during the production procedure, small amounts of locust bean gum hydrocolloids might be present in the fibre fraction of the investigated carob HPI contributing to its high bread quality. Also lipids are known to play an important role in wheat flour doughs. Depending on their nature, they are believed to have a positive effect on crumb softness and to support a finer crumb structure (Pareyt et al., 2011). The fine crumb structure (characterised by a low cell area) observed for the pea bread could therefore be related to the high fat content of this HPI.

5. Conclusion

This study provides a comparative evaluation of HPIs from different plant sources applied in wheat bread formulations. The baking performance of a formulation mainly depends on the rheological properties of the dough and its behaviour during heat treatment and subsequent cooling. In plant-based HPI formulations, the proteins were found to affect both. An accelerated and weakened gluten-aggregation was observed for pea and potato. GlutoPeak results further suggest secondary network-formation between non-wheat proteins, especially carob and zein, and gluten. Due to heat induced gelation of non-wheat proteins, dough characteristics can undergo major changes during the baking process and potentially improve baking performance as observed for the potato formulation. The overall quality of the potato formulation is very similar to the control as visualised by PCA. Zein and carob, with dough characteristics very different to the control, lead to improved bread quality. Amongst the remaining legume formulations (pea, lupin, faba bean), especially the final quality of the faba bean bread is remarkable, since this HPI was produced without a wet protein isolation step and therefore promises better sustainability. The establishment of a generic rule as to how HPIs influence final bread quality is difficult. Many factors play an important role and might even oppose each other's impact on the bread formulation. Key properties of HPIs were found to be gelling behaviour upon heat treatment, the ability of co-networking with gluten, the degree of gluten-network impairment and the extent of the presence of other, potentially techno-functional, minor components. Particularly the legume formulations, which promise balanced amino acid profiles for human nutrition, should be in the focus of future studies.

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Tables

Table 1: Recipe for control and HPI formulations

Ingredient	% based on flour	% based on recipe
Wheat flour	100.0 (85.0**)	59.70
HPI	0.0 (15.0**)	0.00
Baker's yeast	2.0	1.19
NaCl	2.0	37.31
Oil	1.0	1.19
Water	62.5 (FWA*/**)	0.60
Total	167.5	100.00

* FWA - Farinograph water absorption according to Table 3
** for HPI formulations

Table 2: Composition of wheat flour and HPIs

Component	Wheat flour/control	Gluten	Zein	Potato	Carob	Pea	Lupin	Faba bean
Moisture [%]	13.01 ± 0.05^{f}	8.20 ± 0.02^{c}	6.44 ± 0.08^{b}	10.87 ± 0.02^{e}	6.06 ± 0.02^{a}	9.73 ± 0.02^{d}	6.45 ± 0.06^{b}	13.07 ± 0.12^{f}
Protein [%DM]	14.09 ± 0.00^{a}	83.11 ± 0.42^{e}	91.79 ± 0.38^{f}	94.06 ± 2.13^{fg}	55.04 ± 0.28^{b}	80.19 ± 1.43^{d}	94.51 ± 1.50^{g}	61.25 ± 0.66^{c}
Fat [%DM]	1.97 ± 0.10^{bcd}	0.72 ± 0.02^{ab}	2.66 ± 0.12^{cde}	0.12 ± 0.07^{a}	0.20 ± 0.02^{a}	6.45 ± 0.52^{f}	2.94 ± 0.13^{de}	3.81 ± 0.13^{e}
Ash [%DM]	1.09 ± 0.01^{c}	0.87 ± 0.01^{b}	1.16 ± 0.09^{d}	0.05 ± 0.04^{a}	7.04 ± 0.03^{h}	5.90 ± 0.01^{g}	5.62 ± 0.01^{f}	5.43 ± 0.03^{e}
Total dietary fibre (TDF) [%DM]	2.30	<0.1	0.75	<0.1	17.67	2.88	<0.1	0.35
Carbohydrates* [%DM]	80.56	15.31	3.19	5.76	20.05	4.58	0.00	29.17
Total starch [%DM]	72.38 ± 0.10^{c}	4.95 ± 0.27^{a}	<0.2	<0.2	<0.2	<0.2	<0.2	7.77 ± 0.02^{b}
proteinE (formulation)** [%]	14.48	24.78	26.23	26.13	21.23	24.19	26.64	21.24

Means ± standard deviation with different letters in the same row were significantly different ($p < 0.05$).

* calculated by subtraction, required for calculation of proteinE of formulation

** calculated based on compositional analysis of ingredients

Table 3: Properties of wheat flour and HPI/flour blends and quality characteristics of control and HPI bread formulations

Variable	Wheat flour/control	Gluten	Zein	Potato	Carob	Pea	Lupin	Faba bean
Farinograph								
Farinograph water absorption (FWA) [%]	63.0 ± 0.5^{b}	70.2 ± 0.2^{d}	60.8 ± 0.2^{a}	65.2 ± 0.3^{c}	69.8 ± 0.6^{d}	71.7 ± 0.6^{e}	66.2 ± 0.3^{c}	62.2 ± 0.3^{b}
GlutoPeak								
Peak maximum time (PMT) [s]	53 ± 2^{e}	n.d.	105 ± 4^{f}	17 ± 3^{a}	41 ± 1^{d}	25 ± 1^{b}	33 ± 1^{c}	34 ± 1^{c}
Torque maximum (TM) [BE]	74 ± 1^{e}	n.d.	48 ± 1^{a}	54 ± 0^{b}	58 ± 1^{c}	72 ± 3^{e}	59 ± 1^{c}	51 ± 2^{ab}
Rapid Visco Analyser								
Peak viscosity (PV) [cP]	2390 ± 13^{e}	1683 ± 12^{c}	1415 ± 39^{a}	2365 ± 44^{e}	1540 ± 14^{b}	1518 ± 12^{b}	1500 ± 15^{b}	1852 ± 28^{d}
Setback [cP]	1282 ± 7^{f}	848 ± 9^{c}	877 ± 30^{c}	1058 ± 10^{d}	791 ± 17^{b}	680 ± 6^{a}	1161 ± 4^{e}	1158 ± 3^{e}
Final viscosity (FV) [cP]	2544 ± 10^{e}	1783 ± 4^{c}	1689 ± 52^{b}	2192 ± 22^{d}	1665 ± 35^{b}	1589 ± 19^{a}	2162 ± 15^{d}	2200 ± 17^{d}
Rheometer								
Damping factor (DF)	0.401 ± 0.004^{c}	0.385 ± 0.005^{c}	0.387 ± 0.004^{c}	0.437 ± 0.016^{d}	0.327 ± 0.005^{a}	0.325 ± 0.002^{a}	0.349 ± 0.001^{b}	0.329 ± 0.002^{a}
Bread analysis								
Specific volume (SV) [ml/g]	2.55 ± 0.20^{bc}	3.81 ± 0.05^{d}	2.63 ± 0.06^{bc}	2.81 ± 0.23^{c}	2.73 ± 0.34^{bc}	2.00 ± 0.08^{a}	1.98 ± 0.03^{a}	2.26 ± 0.08^{ab}
Number of cells	1671 ± 47^{ab}	1902 ± 57^{b}	1835 ± 73^{ab}	1811 ± 240^{ab}	1709 ± 105^{ab}	1708 ± 92^{ab}	1596 ± 91^{ab}	1543 ± 115^{a}
Cell area [%]	45.5 ± 0.8^{ab}	49.0 ± 0.2^{c}	46.3 ± 0.3^{ab}	46.6 ± 0.2^{b}	46.0 ± 1.5^{ab}	44.5 ± 0.7^{a}	45.0 ± 0.6^{ab}	46.6 ± 0.5^{b}
Hardness [N]	11.81 ± 1.62^{bc}	4.12 ± 0.45^{a}	15.10 ± 1.47^{cd}	19.02 ± 4.80^{d}	7.84 ± 1.08^{ab}	16.68 ± 1.24^{cd}	20.11 ± 1.14^{d}	16.30 ± 3.08^{cd}
Lightness of crust (L*)	71.84 ± 3.48^{de}	66.48 ± 0.23^{bc}	72.80 ± 1.26^{e}	70.58 ± 0.84^{cde}	62.33 ± 2.52^{ab}	66.83 ± 0.64^{bcd}	64.18 ± 0.79^{b}	57.06 ± 2.62^{a}

Means ± standard deviation with different letters in the same row were significantly different at ($p < 0.05$).
n.d. - not detectable (analysis could not be performed due to exceeded maximum torque of the device)

Figures

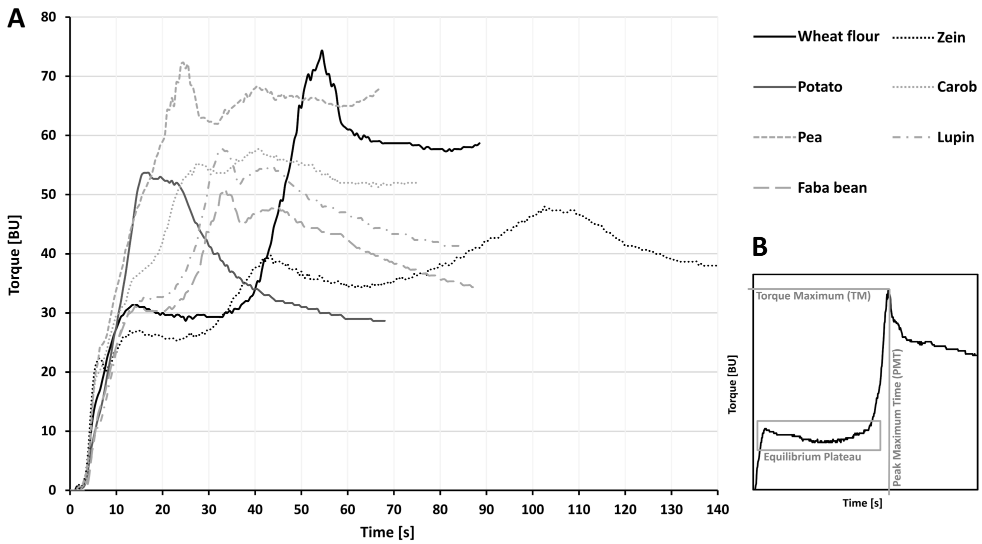

Figure 1: A - GlutoPeak curves of wheat flour and HPI/flour blends; B - schematic GlutoPeak curve of wheat flour with explanation of associated variables and terms

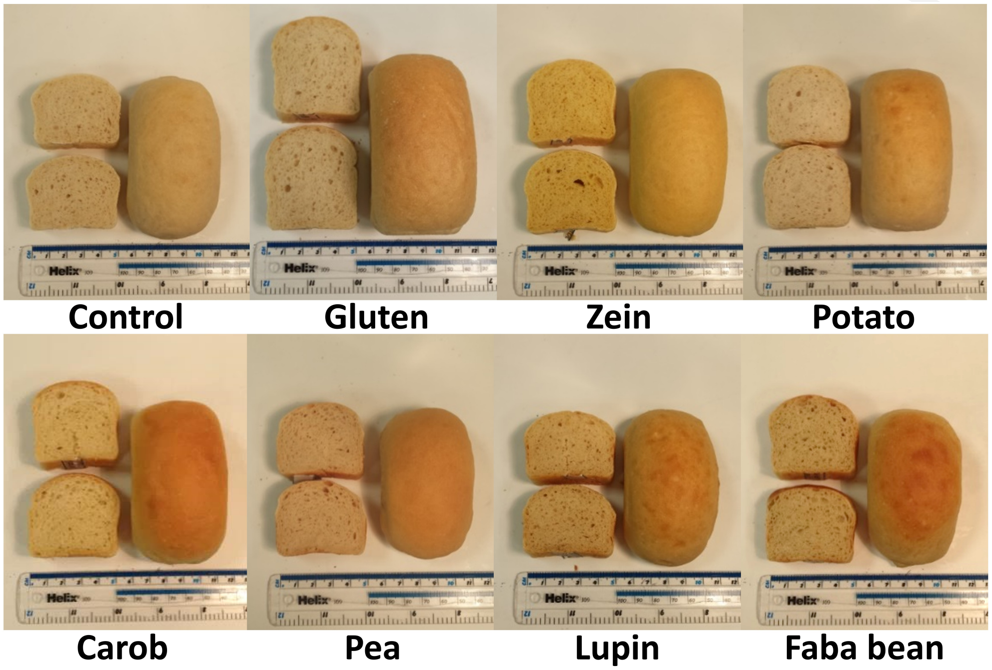

Figure 2: Appearance of loaves and slices of control and HPI breads

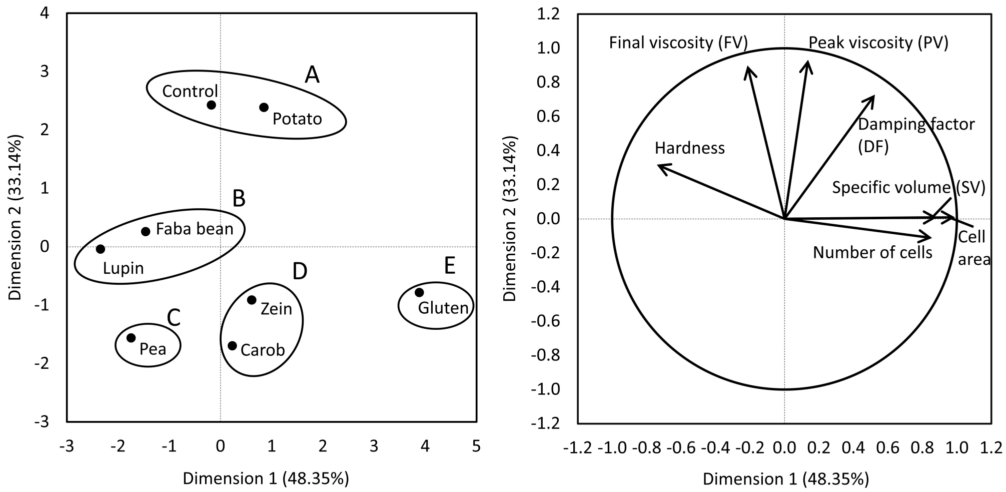

Figure 3: Principal component analysis of dough and bread characteristics: left - analysed observations with hierarchical classification, right - analysed variables